FIGURE 1

cDNA sequence of wild type amFP486

ATGGCTCTTTCAAACAAGTTTATCGGAGATGACATGAAAATGACCTACCATATGGATG
GCTGTGTCAATGGGCATTACTTTACCGTCAAAGGTGAAGGCAACGGGAAGCCATACGA
AGGGACGCAGACCTCGACTTTTAAAGTCACCATGGCCAACGGTGGGCCCCTTGCATTC
TCCTTTGACATACTATCTACAGTGTTCAAGTATGGAAATCGATGCTTTACTGCGTATC
CTACCAGTATGCCCGACTATTTCAAACAAGCATTTCCTGACGGAATGTCATATGAAAG
GACTTTTACCTATGAAGATGGAGGAGTTGCTACAGCCAGTTGGGAAATAAGCCTTAAA
GGCAACTGCTTTGAGCACAAATCCACGTTTCATGGAGTGAACTTTCCTGCTGATGGAC
CTGTGATGGCGAAGATGACAACTGGTTGGGACCCATCTTTTGAGAAAATGACTGTCTG
CGATGGAATATTGAAGGGTGATGTCACCGCGTTCCTCATGCTGCAAGGAGGTGGCAAT
TACAGATGCCAATTCCACACTTCTTACAAGACAAAAAAACCGGTGACGATGCCACCAA
ACCATGCGGTGGAACATCGCATTGCGAGGACCGACCTTGACAAAGGTGGCAACAGTGT
TCAGCTGACGGAGCACGCTGTTGCACATATAACCTCTGTTGTCCCTTTC (SEQ ID
NO:01)

amino acid sequence of wild type amFP486

MALSNKFIGD DMKMTYHMDG CVNGHYFTVK GEGNGKPYEG TQTSTFKVTM ANGGPLAFSF
DILSTVFKYG NRCFTAYPTS MPDYFKQAFP DGMSYERTFT YEDGGVATAS WEISLKGNCF
EHKSTFHGVN FPADGPVMAK MTTGWDPSFE KMTVCDGILK GDVTAFLMLQ GGGNYRCQFH
TSYKTKKPVT MPPNHAVEHR IARTDLDKGG NSVQLTEHAV AHITSVVPF
(SEQ ID NO:02)

Figure 2

cDNA sequence of zFP506

ATGGCTCAGTCAAAGCACGGTCTAACAAAAGAAATGACAATGAAATACCGTATGGAAGGGTGC
GTCGATGGACATAAATTTGTGATCACGGGAGAGGGCATTGGATATCCGTTCAAAGGGAAACAG
GCTATTAATCTGTGTGTGGTCGAAGGTGGACCATTGCCATTTGCCGAAGACATATTGTCAGCT
GCCTTTATGTACGGAAACAGGGTTTTCACTGAATATCCTCAAGACATAGCTGACTATTTCAAG
AACTCGTGTCCTGCTGGTTATACATGGGACAGGTCTTTTCTCTTTGAGGATGGAGCAGTTTGC
ATATGTAATGCAGATATAACAGTGAGTGTTGAAGAAAACTGCATGTATCATGAGTCCAAATTT
TATGGAGTGAATTTTCCTGCTGATGGACCTGTGATGAAAAAGATGACAGATAACTGGGAGCCA
TCCTGCGAGAAGATCATACCAGTACCTAAGCAGGGGATATTGAAAGGGGATGTCTCCATGTAC
CTCCTTCTGAAGGATGGTGGGCGTTTACGGTGCCAATTCGACACAGTTTACAAAGCAAAGTCT
GTGCCAAGAAAGATGCCGGACTGGCACTTCATCCAGCATAAGCTCACCCGTGAAGACCGCAGC
GATGCTAAGAATCAGAAATGGCATCTGACAGAACATGCTATTGCATCCGGATCTGCATTGCCC
(SEQ ID NO:03)

amino acid sequence of zFP506

MAQSKHGLTK EMTMKYRMEG CVDGHKFVIT GEGIGYPFKG KQAINLCVVE GGPLPFAEDI LSAAFNYGNR VFTEYPQDIA
DYFKNSCPAG YTWDRSFLFE DGAVCICNAD ITVSVEENCM YHESKFYGVN FPADGPVMKK MTDNWEPSCE KIIPVPKQGI
LKGDVSMYLL LKDGGRLRCQ FDTVYKAKSV PRKMPDWHFI QHKLTREDRS DAKNQKWHLT EHAIASGSAL P
(SEQ ID NO:04)

Figure 3

cDNA sequence of zFP538

```
gagttgagtt tctcgacttc agttgtatca attttggggc atcaagcgat ctattttcaa
catggctcat tcaaagcacg gtctaaaaga agaaatgaca atgaaatacc acatggaagg
gtgcgtcaac ggacataaat ttgtgatcac gggcgaaggc attggatatc cgttcaaagg
gaaacagact attaatctgt gtgtgatcga agggggacca ttgccatttt ccgaagacat
attgtcagct ggctttaagt acggagacag gattttcact gaatatcctc aagacatagt
agactatttc aagaactcgt gtcctgctgg atatacatgg ggcaggtctt ttctctttga
ggatggagca gtctgcatat gcaatgtaga tataacagtg agtgtcaaag aaaactgcat
ttatcataag agcatattta atggaatgaa ttttcctgct gatggacctg tgatgaaaaa
gatgacaact aactgggaag catcctgcga gaagatcatg ccagtaccta agcaggggat
actgaaaggg gatgtctcca tgtacctcct tctgaaggat ggtgggcgtt accggtgcca
gttcgacaca gtttacaaag caaagtctgt gccaagtaag atgccggagt ggcacttcat
ccagcataag ctcctccgtg aagaccgcag cgatgctaag aatcagaagt ggcagctgac
agagcatgct attgcattcc cttctgcctt ggcctgataa gaatgtagtt ccaacatttt
aatgcatgtg cttgtcaatt attctgataa aaatgtagtt gagttgaaaa cagacaagta
caaataaagc acatgtaaat cgtct        (SEQ ID NO:05)
```

amino acid sequence of zFP538

```
Met Ala His Ser Lys His Gly Leu Lys Glu Glu Met Thr Met Lys
Tyr His Met Glu Gly Cys Val Asn Gly His Lys Phe Val Ile Thr
Gly Glu Gly Ile Gly Tyr Pro Phe Lys Gly Lys Gln Thr Ile Asn
Leu Cys Val Ile Glu Gly Gly Pro Leu Pro Phe Ser Glu Asp Ile
Leu Ser Ala Gly Phe Lys Tyr Gly Asp Arg Ile Phe Thr Glu Tyr
Pro Gln Asp Ile Val Asp Tyr Phe Lys Asn Ser Cys Pro Ala Gly
Tyr Thr Trp Gly Ser Phe Leu Phe Glu Asp Gly Ala Val Cys Ile
Cys Asn Val Asp Ile Thr Val Ser Val Lys Glu Asn Cys Ile Tyr
His Lys Ser Ile Phe Asn Gly Met Asn Phe Pro Ala Asp Gly Pro
Val Met Lys Lys Met Thr Thr Asn Trp Glu Ala Ser Cys Glu Lys
Ile Met Pro Val Pro Lys Gln Gly Ile Leu Lys Gly Asp Val Ser
Met Tyr Leu Leu Leu Lys Asp Gly Gly Arg Tyr Arg Cys Gln Phe
Asp Thr Val Tyr Lys Ala Lys Ser Val Pro Ser Lys Met Pro Glu
Trp His Phe Ile Gln His Lys Leu Leu Arg Glu Asp Arg Ser Asp
Ala Lys Asn Gln Lys Trp Gln Leu Thr Glu His Ala Ile Ala Phe
Pro Ser Ala Leu Ala (SEQ ID NO:06)
```

FIGURE 4

All mutants are derived from drFP583 (called "pink" or FP6.) by random mutagenesis

The mutants **E57** and **AG4** are derivative from **E5**

Mutant: **E5 = V105A, S197T** Phenotype: in *E.coli* seen as Green overnight, matures to Red over 24h at 37°C (final peaks ratio Red vs. Green is 75:25); folding is faster then **FP6**.

Mutant: **E8 = N42H** Phenotype: always has two peaks Green & Red in approx. 60:40; folding is faster than **E5** (about 8h at 37°C)

Mutant: **E83 = N42H, V71A, I180V** Phenotype: always has two almost equal peaks Green & Red; folding is the same as for **E8**

Mutant: **E5up = V105A** Phenotype: seen as Red from the beginning; folding is faster than **E5** (about 12-16h) Almost no Green peak at final point of maturation

Mutant: **E57 = V105A, I161T, S197A** Phenotype: at common is like **E5up** but folding is more faster (no more that 8-10h) Very small Green peak at final point of maturation (less that 5%)

Mutant: **E5down = S197T** Phenotype and folding rate are exactly the same as for E5

Mutant: **AG4 = V71M, V105A, S197T** Phenotype: Very bright Green, no Red at all (even at the beginning); folding is faster than **E5** (no more that 16h)

Mutant: **AG4 = V71M, V105A, Y120H, S197T** Phenotype: at common is like **AG4**, but more brighter (appox. twice) one.

```
     1  Met Arg Ser Ser Lys Asn Val Ile Lys Glu Phe Met Arg Phe Lys Val      16
     1  ATG CGC TCC TCC AAG AAC GTC ATC AAG GAG TTC ATG CGC TTC AAG GTG      48

    17  Arg Met Glu Gly Thr Val Asn Gly His Glu Phe Glu Ile Glu Gly Glu      32
    49  CGC ATG GAG GGC ACC GTG AAC GGC CAC GAG TTC GAG ATC GAG GGC GAG      96

                                              His(CAC) for E8 and E83
    33  Gly Glu Gly Arg Pro Tyr Glu Gly His Asn Thr Val Lys Leu Lys Val      48
    97  GGC GAG GGC CGC CCC TAC GAG GGC CAC AAC ACC GTG AAG CTG AAG GTG     144

    49  Thr Lys Gly Gly Pro Leu Pro Phe Ala Trp Asp Ile Leu Ser Pro Gln      64
   145  ACC AAG GGC GGC CCC CTG CCC TTC GCC TGG GAC ATC CTG TCC CCC CAG     192

                                    Met(ATG) for AG4 and AG45/Ala(GCG) for E83
    65  Phe Gln Tyr Gly Ser Lys Val Tyr Val Lys His Pro Ala Asp Ile Pro      80
   193  TTC CAG TAC GGC TCC AAG GTG TAC GTG AAG CAC CCC GCC GAC ATC CCC     240

    81  Asp Tyr Lys Lys Leu Ser Phe Pro Glu Gly Phe Lys Trp Glu Arg Val      96
   241  GAC TAC AAG AAG CTG TCC TTC CCC GAG GGC TTC AAG TGG GAG CGC GTG     288

                                          Ala(GCG)-for E5, E57, AG4 and AG45
    97  Met Asn Phe Glu Asp Gly Gly Val Val Thr Val Thr Gln Asp Ser Ser     112
   289  ATG AAC TTC GAG GAC GGC GGC GTG GTG ACC GTG ACC CAG GAC TCC TCC     336

                                        His(CAC)-for AG45
   113  Leu Gln Asp Gly Cys Phe Ile Tyr Lys Val Lys Phe Ile Gly Val Asn     128
   337  CTG CAG GAC GGC TGC TTC ATC TAC AAG GTG AAG TTC ATC GGC GTG AAC     384

   129  Phe Pro Ser Asp Gly Pro Val Met Gln Lys Lys Thr Met Gly Trp Glu     144
   385  TTC CCC TCC GAC GGC CCC GTG ATG CAG AAG AAG ACC ATG GGC TGG GAG     432

   145  Ala Ser Thr Glu Arg Leu Tyr Pro Arg Asp Gly Val Leu Lys Gly Glu     160
   433  GCC TCC ACC GAG CGC CTG TAC CCC CGC GAC GGC GTG CTG AAG GGC GAG     480

        Thr(ACC) for E57
   161  Ile His Lys Ala Leu Lys Leu Lys Asp Gly Gly His Tyr Leu Val Glu     176
   481  ATC CAC AAG GCC CTG AAG CTG AAG GAC GGC GGC CAC TAC CTG GTG GAG     528

                    Val(GTC) for E83
   177  Phe Lys Ser Ile Tyr Met Ala Lys Lys Pro Val Gln Leu Pro Gly Tyr     192
   529  TTC AAG TCC ATC TAC ATG GCC AAG AAG CCC GTG CAG CTG CCC GGC TAC     576

                        Thr(ACC) for E5, AG4 and AG45/Ala(GCC) for E57
   193  Tyr Tyr Val Asp Ser Lys Leu Asp Ile Thr Ser His Asn Glu Asp Tyr     208
   577  TAC TAC GTG GAC TCC AAG CTG GAC ATC ACC TCC CAC AAC GAG GAC TAC     624

209 Thr Ile Val Glu Gln Tyr Glu Arg Thr Glu Gly Arg His His Leu Phe Leu *** 229
625 ACC ATC GTG GAG CAG TAC GAG CGC ACC GAG GGC CGC CAC CAC CTG TTC CTG TAA 678
(SEQ ID NO:07 & 8)
```

FIGURE 5

Amino Acid and Nucleotide Sequence for asFP600

ATGGCTTCCTTTTTAAAGAAGACTATGCCCTTTAAGACGACCATTGAAGGGACGGTTAATGGCCAC
TACTTCAAGTGTACAGGAAAAGGAGAGGGCAACCCATTTGAGGGTACGCAGGAAATGAAGATAGAG
GTCATCGAAGGAGGTCCATTGCCATTTGCCTTCCACATTTTGTCAACGAGTTGTATGTACGGTAGT
AAGGCCTTCATCAAGTATGTGTCAGGAATTCCTGACTACTTCAAGCAGTCTTTCCCTGAAGGTTTT
ACTTGGGAAAGAACCACAACCTACGAGGATGGAGGCTTTCTTACAGCTCATCAGGACACAAGCCTA
GATGGAGATTGCCTCGTTTACAAGGTCAAGATTCTTGGTAATAATTTTCCTGCTGATGGCCCCGTG
ATGCAGAACAAAGCAGGAAGATGGGAGCCATCCACCGAGATAGTTTATGAAGTTGACGGTGTCCTG
CGTGGACAGTCTTTGATGGCCCTTAAGTGCCCTGGTGGTCGTCATCTGACTTGCCATCTCCATACT
ACTTACAGGTCCAAAAAACCAGCTGCTGCCTTGAAGATGCCAGGATTTCATTTTGAAGATCATCGC
ATCGAGATAATGGAGGAAGTTGAGAAAGGCAAGTGCTATAAACAGTACGAAGCAGCAGTGGGCAGG
TACTGTGATGCTGCTCCATCCAAGCTTGGACATAAC (SEQ ID NO:09)

Amino acid

MASFLKKTMP FKTTIEGTVN GHYFKCTGKG EGNPFEGTQE MKIEVIEGGP LPFAFHILST
SCMYGSKTFI KYVSGIPDYF KQSFPEGFTW ERTTTYEDGG FLTAHQDTSL DGDCLVYKVK
ILGNNFPADG PVMQNKAGRW EPATEIVYEV DGVLRGQSLM ALKCPGGRHL TCHLHTTYRS
KKPAAALKMP GFHFEDHRIE IMEEVEKGKC YKQYEAAVGR YCDAAPSKLG HN (SEQ ID
NO:10)

Figure 6

Sequence of humanized 6/9 hybrid gene and 6/9-Q3 mutant

for *6/9-2G* and *6/9-Q3* CAG(Q)

```
   1  ATG AGC TGC AGC AAG AAC GTG ATC AAG GAG TTC ATG CGG TTC AAG GTG   48
   1   M   S   C   S   K   N   V   I   K   E   F   M   R   F   K   V   16

  49  CGG ATG GAG GGC ACC GTG AAC GGC CAC GAG TTC GAG ATC AAG GGC GAG   96
  17   R   M   E   G   T   V   N   G   H   E   F   E   I   K   G   E   32

  97  GGC GAG GGC CGG CCC TAC GAG GGC CAC TGC AGC GTG AAG CTC ATG GTG  144
  33   G   E   G   R   P   Y   E   G   H   C   S   V   K   L   M   V   48

 145  ACC AAG GGC GGC CCC CTC CCC TTC GCC TTC GAC ATC CTC AGC CCC CAG  192
  49   T   K   G   G   P   L   P   F   A   F   D   I   L   S   P   Q   64

 193  TTC CAG TAC GGC AGC AAG GTG TAC GTG AAG CAC CCC GCC GAC ATC CCC  240
  65   F   Q   Y   G   S   K   V   Y   V   K   H   P   A   D   I   P   80

          ATG(M) for 6/9-Q3
 241  GAC TAC AAG AAG CTC AGC TTC CCC GAG GGC TTC AAG TGG GAG CGG GTG  288
  81   D   Y   K   K   L   S   F   P   E   G   F   K   W   E   R   V   96

 289  ATG AAC TTC GAG GAC GGC GGC GTG GTG ACC GTG AGC CAG GAC AGC AGC  336
  97   M   N   F   E   D   G   G   V   V   T   V   S   Q   D   S   S  112

 337  CTC AAG GAC GGC TGC TTC ATC TAC GAG GTG AAG TTC ATC GGC GTG AAC  384
 113   L   K   D   G   C   F   I   Y   E   V   K   F   I   G   V   N  128

 385  TTC CCC AGC GAC GGC CCC GTG ATG CAG CGG CGG ACC CGG GGC TGG GAG  432
 129   F   P   S   D   G   P   V   M   Q   R   R   T   R   G   W   E  144

 433  GCC AGC AGC GAG CGG CTC TAC CCC CGG GAC GGC GTG CTC AAG GGC GAC  480
 145   A   S   S   E   R   L   Y   P   R   D   G   V   L   K   G   D  160

 481  ATC CAC ATG GCC CTC CGG CTC GAG GGC GGC GGC CAC TAC CTC GTG GAG  528
 161   I   H   M   A   L   R   L   E   G   G   G   H   Y   L   V   E  176

 529  TTC AAG AGC ATC TAC ATG GCC AAG AAG CCC GTG CAG CTC CCC GGC TAC  576
 177   F   K   S   I   Y   M   A   K   K   P   V   Q   L   P   G   Y  192

 577  TAC TAC GTG GAC AGC AAG CTC GAC ATC ACC AGC CAC AAC GAG GAC TAC  624
 193   Y   Y   V   D   S   K   L   D   I   T   S   H   N   E   D   Y  208

                          TCC(S) for 6/9-2G and 6/9-Q3
 625  ACC ATC GTG GAG CAG TAC GAG CGG ACC GAG GGC CGG CAC CAC CTC TTC  672
 209   T   I   V   E   Q   Y   E   R   T   E   G   R   H   H   L   F  224

 673  CTC TGA                                                         678
 225   L   *                                                          226
```

(SEQ ID NO:11 & 12)

Figure 7

Nucleic acid sequence FP6 (E57)-NA

ATGGCCTCCTCCGAGAACGTCATCACCGAGTTCATGCGCTTCAAGGTGCGCATGGAGGGCACCGTGA
ACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCCACAACACCGTG
AAGCTGAAGGTGACCAAGGGCGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCCCAGTTCCAGT
ACGGCTCCAAGGTGTACGTGAAGCACCCCGCCGACATCCCCGACTACAAGAAGCTGTCCTTCCCCGA
GGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGCGACCGTGACCCAGGACTC
CTCCCTGCAGGACGGCTGCTTCATCTACAAGGTGAAGTTCATCGGCGTGAACTTCCCCTCCGACGGC
CCCGTGATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCACCGAGCGCCTGTACCCCCGCGACGGC
GTGCTGAAGGGCGAGACCCACAAGGCCCTGAAGCTGAAGGACGGCGGCCACTACCTGGTGGAGTTC
AAGTCCATCTACATGGCCAAGAAGCCCGTGCAGCTGCCCGGCTACTACTACGTGGACGCCAAGCTGG
ACATCACCTCCCACAACGAGGACTACACCATCGTGGAGCAGTACGAGCGCACCGAGGGCCGCCACCA
CCTGTTCCTG (SEQ ID NO:13)

FIgure 8

DNA sequence (ORF) of E5-NA

ATGGCCTCCTCCGAGAACGTCATCACCGAGTTCATGCGCTTCAAGGTGCGCATGGAGGGCACCGTGA
ACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCCACAACACCGTG
AAGTTGAAGGTGACCAAGGGCGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCCCAGTTCCAGT
ACGGCTCCAAGGTGTACGTGAAGCACCCCGCCGACATCCCCGACTACAAGAAGCTGTCCTTCCCCGA
GGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGCGACCGTGACCCAGGACTC
CTCCCTGCAGGACGGCTGCTTCATCTACAAGGTGAAGTTCATCGGCGTGAACTTCCCCTCCGACGGC
CCCGTGATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCACCGAGCGCCTGTACCCCCGCGACGGC
GTGCTGAAGGGCGAGATCCACAAGGCCCTGAAGCTGAAGGACGGCGGCCACTACCTGGTGGAGTTC
AAGTCCATCTACATGGCCAAGAAGCCCGTGCAGCTGCCCGGCTACTACTACGTGGACACCAAGCTGG
ACATCACCTCCCACAACGAGGACTACACCATCGTGGAGCAGTACGAGCGCACCGAGGGCCGCCACCA
CCTGTTCCTGTAA (SEQ ID NO:14)

Figure 9

Non-aggregating mutant FP3-NA was generated from zFP506-N65M (non-humanized version). In comparison with zFP506-N65M, FP3-NA contains two additional amino acid substitutions - K5E and K10E. Also, one accidental nucleotide substitution was introduced due to PCR mistake (double underline).

Cloning into pQE30 was done using BamHI and HindIII sites:

```
GGA TCC GCT CAG TCA GAG CAC GGT CTA ACA GAA GAA ATG ACA ATG AAA
 BamHI   A   Q   S   E   H   G   L   T   E   E   M   T   M   K

TAC CGT ATG GAA GGG TGC GTC GAT GGA CAT AAA TTT GTG ATC ACG GGA
 Y   R   M   E   G   C   V   D   G   H   K   F   V   I   T   G

GAG GGC ATT GGA TAT CCG TTC AAA GGG AAA CAG GCT ATT AAT CTG TGT
 E   G   I   G   Y   P   F   K   G   K   Q   A   I   N   L   C

GTG GTC GAA GGT GGA CCA TTG CCA TTT GCC GAA GAC ATA TTG TCA GCT
 V   V   E   G   G   P   L   P   F   A   E   D   I   L   S   A

GCC TTT ATG TAC GGA AAC AGG GTT TTC ACT GAA TAT CCT CAA GAC ATA
 A   F   M   Y   G   N   R   V   F   T   E   Y   P   Q   D   I

GTT GAC TAT TTC AAG AAC TCG TGT CCT GCT GGA TAT ACA TGG GAC AGG
 V   D   Y   F   K   N   S   C   P   A   G   Y   T   W   D   R

TCT TTT CTC TTT GAG GAT GGA GCA GTT TGC ATA TGT AAT GCA GAT ATA
 S   F   L   F   E   D   G   A   V   C   I   C   N   A   D   I

ACA GTG AGT GTT GAA GAA AAC TGC ATG TAT CAT GAG TCC AAA TTC TAT
 T   V   S   V   E   E   N   C   M   Y   H   E   S   K   F   Y

GGA GTG AAT TTT CCT GCT GAT GGA CCT GTG ATG AAA AAG ATG ACA GAT
 G   V   N   F   P   A   D   G   P   V   M   K   K   M   T   D

AAC TGG GAG CCA TCC TGC GAG AAG ATC ATA CCA GTA CCT AAG CAG GGG
 N   W   E   P   S   C   E   K   I   I   P   V   P   K   Q   G

ATA TTG AAA GGG GAT GTC TCC ATG TAC CTC CTT CTG AAG GAT GGT GGG
 I   L   K   G   D   V   S   M   Y   L   L   L   K   D   G   G

CGT TTA CGG TGC CAA TTC GAC ACA GTT TAC AAA GCA AAG TCT GTG CCA
 R   L   R   C   Q   F   D   T   V   Y   K   A   K   S   V   P

AGA AAG ATG CCG GAC TGG CAC TTC ATC CAG CAT AAG CTC ACC CGT GAA
 R   K   M   P   D   W   H   F   I   Q   H   K   L   T   R   E

GAC CGC AGC GAT GCT AAG AAT CAG AAA TGG CAT CTG ACA GAA CAT GCT
 D   R   S   D   A   K   N   Q   K   W   H   L   T   E   H   A

ATT GCA TCC GGA TCT GCA TTG CCC TGA AAGCTT
 I   A   S   G   S   A   L   P   *  HindIII    (SEQ ID NO:15 & 16)
```

Figure 10

Non-aggregating mutant FP4-NA was generated from zFP538-M128V (humanized version). In comparison with zFP538-M128V, FP4-NA contains two additional amino acid substitutions - K5E and K9T. Also, two accidental nucleotide substitutions were introduced due to PCR mistakes (double underline).

Cloning into pQE30 was done using BamHI and HindIII sites:

```
GGA TCC GCC CAC AGC GAG CAC GGC CTG ACC GAG GAG ATG ACC ATG AAG
 BamHI   A   H   S   E   H   G   L   T   E   E   M   T   M   K

TAC CAC ATG GAG GGC TGC GTG AAC GGC CAC AAG TTC GTG ATC ACC GGC
 Y   H   M   E   G   C   V   N   G   H   K   F   V   I   T   G

GAG GGC ATC GGC TAC CCC TTC AAG GGC AAG CAG ACC ATC AAC CTG TGC
 E   G   I   G   Y   P   F   K   G   K   Q   T   I   N   L   C

GTG ATC GAG GGC GGC CCC CTG CCC TTC AGC GAG GAC ATC CTG AGC GCC
 V   I   E   G   G   P   L   P   F   S   E   D   I   L   S   A

GGC TTC AAG TAC GGC GAC CGG ATC TTC ACC GAG TAC CCC CAG GAC ATC
 G   F   K   Y   G   D   R   I   F   T   E   Y   P   Q   D   I

GTG GAC TAC TTC AAG AAC AGC TGC CCC GCC GGC TAC ACC TGG GGC CGG
 V   D   Y   F   K   N   S   C   P   A   G   Y   T   W   G   R

AGC TTC CTG TTC GAG GAC GGC GCC GTG TGC ATC TGT AAC GTG GAC ATC
 S   F   L   F   E   D   G   A   V   C   I   C   N   V   D   I

ACC GTG AGC GTG AAG GAG AAC TGC ATC TAC CAC AAG AGC ATC TTC AAC
 T   V   S   V   K   E   N   C   I   Y   H   K   S   I   F   N

GGC GTG AAC TTC CCC GCC GAC GGC CCC GTG ATG AAG AAG ATG ACC ACC
 G   V   N   F   P   A   D   G   P   V   M   K   K   M   T   T

AAC TGG GAG GCC AGC TGC GAG AAG ATC ATG CCC GTG CCT AAG CAG GGC
 N   W   E   A   S   C   E   K   I   M   P   V   P   K   Q   G

ATC CTG AAG GGC GAC GTG AGC ATG TAC CTG CTG CTG AAG GAC GGC GGC
 I   L   K   G   D   V   S   M   Y   L   L   L   K   D   G   G

CGG TAC CGG TGC CAG TTC GAC ACC GTG TAC AAG GCC AAG AGC GTG CCC
 R   Y   R   C   Q   F   D   T   V   Y   K   A   K   S   V   P

AGC AAG ATG CCC GAG TGG CAC TTC ATC CAG CAC AAG CTG CTG CGG GAG
 S   K   M   P   E   W   H   F   I   Q   H   K   L   L   R   E

GAC CGG AGC GAC GCC AAG AAC CAG AAG TGG CAG CTG ACC GAG CAC GCC
 D   R   S   D   A   K   N   Q   K   W   Q   L   T   E   H   A

ATC GCC TTC CCC AGC GCC CTG GCC TGA AAGCTT
 I   A   F   P   S   A   L   A   *  HindIII          (SEQ ID NOS: 17 & 18)
```

Figure 11

```
          M   A   L   S   N   E   F   I   G   D   D   M   K   M
676       ATG GCC CTG TCC AAC GAG TTC ATC GGC GAC GAC ATG AAG ATG
          TAC CGG GAC AGG TTG TTC AAG TAG CCG CTG CTG TAC TTC TAC
      T   Y   H   M   D   G   C   V   N   G   H   Y   F   T   V
721   ACC TAC CAC ATG GAC GGC TGC GTG AAC GGC CAC TAC TTC ACC GTG
      TGG ATG GTG TAC CTG CCG ACG CAC TTG CCG GTG ATG AAG TGG CAC
      K   G   E   G   S   G   K   P   Y   E   G   T   Q   T   S
766   AAG GGC GAG GGC AGC GGC AAG CCC TAC GAG GGC ACC CAG ACC TCC
      TTC CCG CTC CCG TCG CCG TTC GGG ATG CTC CCG TGG GTC TGG AGG
      T   F   K   V   T   M   A   N   G   G   P   L   A   F   S
811   ACC TTC AAG GTG ACC ATG GCC AAC GGC GGC CCC CTG GCC TTC TCC
      TGG AAG TTC CAC TGG TAC CGG TTG CCG CCG GGG GAC CGG AAG AGG
      F   D   I   L   S   T   V   F   M   Y   G   N   R   C   F
856   TTC GAC ATC CTG TCC ACC GTG TTC ATG TAC GGC AAC CGC TGC TTC
      AAG CTG TAG GAC AGG TGG CAC AAG TAC ATG CCG TTG GCG ACG AAG
      T   A   Y   P   T   S   M   P   D   Y   F   K   Q   A   F
901   ACC GCC TAC CCC ACC AGC ATG CCC GAC TAC TTC AAG CAG GCC TTC
      TGG CGG ATG GGG TGG TCG TAC GGG CTG ATG AAG TTC GTC CGG AAG
      P   D   G   M   S   Y   E   R   T   F   T   Y   E   D   G
946   CCC GAC GGC ATG TCC TAC GAG AGA ACC TTC ACC TAC GAG GAC GGC
      GGG CTG CCG TAC AGG ATG CTC TCT TGG AAG TGG ATG CTC CTG CCG
      G   V   A   T   A   S   W   E   I   S   L   K   G   N   C
991   GGC GTG GCC ACC GCC AGC TGG GAG ATC AGC CTG AAG GGC AAC TGC
      CCG CAC CGG TGG CGG TCG ACC CTC TAG TCG GAC TTC CCG TTG ACG
      F   E   H   K   S   T   F   H   G   V   N   F   P   A   D
1036  TTC GAG CAC AAG TCC ACC TTC CAC GGC GTG AAC TTC CCC GCC GAC
      AAG CTC GTG TTC AGG TGG AAG GTG CCG CAC TTG AAG GGG CGG CTG
      G   P   V   M   A   K   K   T   T   G   W   D   P   S   F
1081  GGC CCC GTG ATG GCC AAG AAG ACC ACC GGC TGG GAC CCC TCC TTC
      CCG GGG CAC TAC CGG TTC TTC TGG TGG CCG ACC CTG GGG AGG AAG
      E   K   M   T   V   C   D   G   I   L   K   G   D   V   T
1126  GAG AAG ATG ACC GTG TGC GAC GGC ATC TTG AAG GGC GAC GTG ACC
      CTC TTC TAC TGG CAC ACG CTG CCG TAG AAC TTC CCG CTG CAC TGG
      A   F   L   M   L   Q   G   G   G   N   Y   R   C   Q   F
1171  GCC TTC CTG ATG CTG CAG GGC GGC GGC AAC TAC AGA TGC CAG TTC
      CGG AAG GAC TAC GAC GTC CCG CCG CCG TTG ATG TCT ACG GTC AAG
      H   T   S   Y   K   T   K   K   P   V   T   M   P   P   N
1216  CAC ACC TCC TAC AAG ACC AAG AAG CCC GTG ACC ATG CCC CCC AAC
      GTG TGG AGG ATG TTC TGG TTC TTC GGG CAC TGG TAC GGG GGG TTG
      H   V   V   E   H   R   I   A   R   T   D   L   D   K   G
1261  CAC GTG GTG GAG CAC CGC ATC GCC AGA ACC GAC CTG GAC AAG GGC
      GTG CAC CAC CTC GTG GCG TAG CGG TCT TGG CTG GAC CTG TTC CCG
      G   N   S   V   Q   L   T   E   H   A   V   A   H   I   T
1306  GGC AAC AGC GTG CAG CTG ACC GAG CAC GCC GTG GCC CAC ATC ACC
      CCG TTG TCG CAC GTC GAC TGG CTC GTG CGG CAC CGG GTG TAG TGG
      S   V   V   P   F   *
1351  TCC GTG GTG CCC TTC TGA
      AGG CAC CAC GGG AAG ACT      (SEQ ID NO:19 & 20)
```

Figure 12

Non-aggregating mutant FP7-NA was generated from M35-5 (FP7a). In comparison with M35-5, FP7-NA contains two additional substitutions - K6T and K7E. Nucleotide substitutions in the codon for Leu-4 were introduced to optimize codon usage (double underline).

Cloning into pQE30 was done using BamHI and HindIII sites:

```
GGA TCC GCC TCC CTG CTG ACC GAG ACC ATG CCC TTC AGG ACC ACC ATC
 BamHI   A   S   L   L   T   E   T   M   P   F   R   T   T   I

GAG GGC ACC GTG AAC GGC CAC TAC TTC AAG TGC ACC GGC AAG GGC GAG
 E   G   T   V   N   G   H   Y   F   K   C   T   G   K   G   E

GGC AAC CCC CTC GAG GGC ACC CAG GAG ATG AAG ATC GAG GTG ATC GAG
 G   N   P   L   E   G   T   Q   E   M   K   I   E   V   I   E

GGC GGC CCC CTG CCC TTC GCC TTC CAC ATC CTG TCC ACC TCC TGC ATG
 G   G   P   L   P   F   A   F   H   I   L   S   T   S   C   M

TAC GGC TCC AAG GCC TTC ATC AAG TAC GTG TCC GGC ATC CCC GAC TAC
 Y   G   S   K   A   F   I   K   Y   V   S   G   I   P   D   Y

TTC AAG CAG TCC CTC CCC GAG GGC TTC ACC TGG GAG CGC ACC ACC ACC
 F   K   Q   S   L   P   E   G   F   T   W   E   R   T   T   T

TAC GAG GAC GGC GGC TTC CTG ACC GCC CAC CAG GAC ACC TCC CTG GAC
 Y   E   D   G   G   F   L   T   A   H   Q   D   T   S   L   D

GGC GAC TGC CTG GTG TAC AAG GTG AAG ATC CTG GGC AAC AAC TTC CCC
 G   D   C   L   V   Y   K   V   K   I   L   G   N   N   F   P

GCC GAC GGC CCC GTG ATG CAG AAC AAG GCC GGC CGC TGG GAG CCC TCC
 A   D   G   P   V   M   Q   N   K   A   G   R   W   E   P   S

ACC GAG ATC GTG TAC GAG GTG GAC GGC GTG CTG CGC GGC CAG TCC CTG
 T   E   I   V   Y   E   V   D   G   V   L   R   G   Q   S   L

ATG GCC CTG GAG TGC CCC GGC GGT CGC CAC CTG ACC TGC CAC CTG CAC
 M   A   L   E   C   P   G   G   R   H   L   T   C   H   L   H

ACC ACC TAC CGC TCC AAG AAG CCC GCC TCC GCC CTG AAG ATG CCC GGC
 T   T   Y   R   S   K   K   P   A   S   A   L   K   M   P   G

TTC CAC TTC GAG GAC CAC CGC ATC GAG ATC CTG GAG GAG GTG GAG AAG
 F   H   F   E   D   H   R   I   E   I   L   E   E   V   E   K

GGC AAG TGC TAC AAG CAG TAC GAG GCC GCC GTG GGC CGC TAC TGC GAC
 G   K   C   Y   K   Q   Y   E   A   A   V   G   R   Y   C   D

GCC GCC CCC TCC AAG CTG GGC CAC AAC TGAAGCTT
 A   A   P   S   K   L   G   H   N   * HindIII (SEQ ID NO:21 & 22)
```

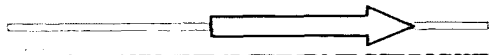
Figure 13

Dimeric mutant AsRed M35-5D (in comparison to M355NA it carries one amino acid substitution L166S according to GFP numbering; L159S according to self-numbering)

```
    ATG GCC TCC CTG CTG ACC GAG ACC ATG CCC TTC AGG ACC ACC ATC
     M   A   S   L   L   T   E   T   M   P   F   R   T   T   I

GAG GGC ACC GTG AAC GGC CAC TAC TTC AAG TGC ACC GGC AAG GGC GAG
 E   G   T   V   N   G   H   Y   F   K   C   T   G   K   G   E

GGC AAC CCC CTC GAG GGC ACC CAG GAG ATG AAG ATC GAG GTG ATC GAG
 G   N   P   L   E   G   T   Q   E   M   K   I   E   V   I   E

GGC GGC CCC CTG CCC TTC GCC TTC CAC ATC CTG TCC ACC TCC TGC ATG
 G   G   P   L   P   F   A   F   H   I   L   S   T   S   C   M

TAC GGC TCC AAG GCC TTC ATC AAG TAC GTG TCC GGC ATC CCC GAC TAC
 Y   G   S   K   A   F   I   K   Y   V   S   G   I   P   D   Y

TTC AAG CAG TCC CTC CCC GAG GGC TTC ACC TGG GAG CGC ACC ACC ACC
 F   K   Q   S   L   P   E   G   F   T   W   E   R   T   T   T

TAC GAG GAC GGC GGC TTC CTG ACC GCC CAC CAG GAC ACC TCC CTG GAC
 Y   E   D   G   G   F   L   T   A   H   Q   D   T   S   L   D

GGC GAC TGC CTG GTG TAC AAG GTG AAG ATC CTG GGC AAC AAC TTC CCC
 G   D   C   L   V   Y   K   V   K   I   L   G   N   N   F   P

GCC GAC GGC CCC GTG ATG CAG AAC AAG GCC GGC CGC TGG GAG CCC TCC
 A   D   G   P   V   M   Q   N   K   A   G   R   W   E   P   S

ACC GAG ATC GTG TAC GAG GTG GAC GGC GTG CTG CGC GGC CAG TCC AGC
 T   E   I   V   Y   E   V   D   G   V   L   R   G   Q   S   S

ATG GCC CTG GAG TGC CCC GGC GGT CGC CAC CTG ACC TGC CAC CTG CAC
 M   A   L   E   C   P   G   G   R   H   L   T   C   H   L   H

ACC ACC TAC CGC TCC AAG AAG CCC GCC TCC GCC CTG AAG ATG CCC GGC
 T   T   Y   R   S   K   K   P   A   S   A   L   K   M   P   G

TTC CAC TTC GAG GAC CAC CGC ATC GAG ATC CTG GAG GAG GTG GAG AAG
 F   H   F   E   D   H   R   I   E   I   L   E   E   V   E   K

GGC AAG TGC TAC AAG CAG TAC GAG GCC GCC GTG GGC CGC TAC TGC GAC
 G   K   C   Y   K   Q   Y   E   A   A   V   G   R   Y   C   D

GCC GCC CCC TCC AAG CTG GGC CAC AAC TGA
 A   A   P   S   K   L   G   H   N   *
```

(SEQ ID NO:23 & 24)

Figure 14



```
MRSSKNVIKEFMRFKVRMEGTVNGHE   drFP583
MSCSKNVIKEFMRFQVRMEGTVNGHE   ds/drFP616
MAQSKHGLTKEMTMKYRMEGCVDGHK   zFP506
MAHSKHGLKEEMTMKYHMEGCVNGHK   zFP538
MALSNKFIGDDMKMTYHMDGCVNGHY   amFP486
   MASFLKKTMPFKTTIEGTVNGHY   asFP595
```

(SEQ ID NOS:25-30)

Figure 15

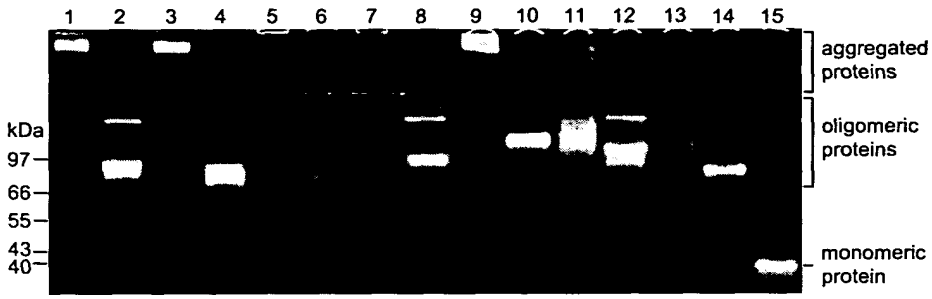
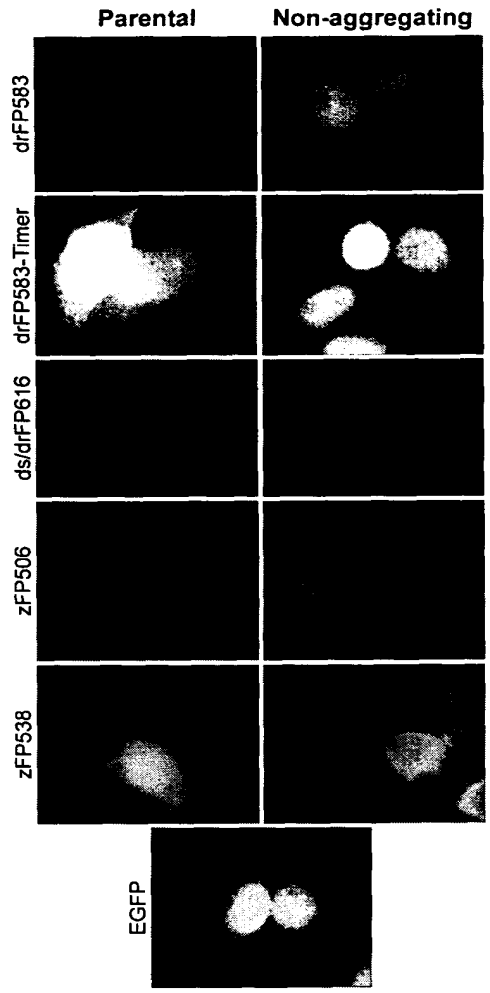

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
kDa
97
66
55
43
40
aggregated proteins
oligomeric proteins
monomeric protein

Figure 16


Parental
Non-aggregating
drFP583
drFP583-Timer
ds/drFP616
zFP506
zFP538
EGFP